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Investigation of thiol/disulfide homeostasis in familial mediterranean fever patients

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Abstract

Aim: To determine the thiol/disulfide homeostasis in patients with familial Mediterranean fever (FMF) and its correlation with the levels of inflammatory markers consisting of white blood cell count, erythrocyte sedimentation rate, C-reactive protein and fibrinogen. Material and Method: This study was performed in the internal Medicine department of Mustafa Kemal University Hospital in Turkey. A total of 27 FMF patients in the attack period (AP), 33 FMF patients in the attack-free period (AFP), and 36 healthy controls participated in this study. Thiol/disulfide profile parameters were detected using the novel method of Erel and Neselioglu. Results: Total and native thiol levels of the FMF-AP group were markedly lower than those of healthy controls, while the difference in disulfide level was not statistically significant. Thiol/disulfide levels in the FMF-AFP group were similar to the levels in both the FMF-AP group and healthy controls. Correlation analysis showed a negative correlation between fibrinogen levels and total and native thiol levels, while there was a positive correlation between white blood cell count and disulfide levels in the FMF-AP group. Discussion: The findings suggest that decreased concentrations of total and native thiol in patients with FMF-AP are likely to be an outcome of inflammation-induced oxidative stress.

Keywords

Familial Mediterranean Fever; Thiol; Disulfide; Oxidative Stress; Inflammation

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Introduction

Familial Mediterranean fever (FMF) is an inherited autoinflammatory disease characterized by periodic attacks of fever with serositis, synovitis, and skin rash. This disease is commonly observed in Jewish, Armenian, Arab, and Turkish societies [1,2]. Although the etiopathogenesis of FMF is not fully understood, Mediterranean Fever (MEFV) gene mutation is known to have an important role. The MEVF gene encodes the pyrin/marenostrin protein. The mutation of MEVF in FMF causes the loss of pyrin function, which results in excessive production of interleukin-1β and dysregulation of leukocyte apoptosis that leads to uncontrolled inflammation [3,4]. During the inflammation process, excessive free radical production occurs [5]. Oxidative stress (OS) caused by impaired balance between free radicals and antioxidants has been reported to increase in patients with FMF [6-8]. Thiols are biologically important organic compounds that include the sulfhydryl group. Thiols can be converted to reversible disulfide bonds through oxidation by reactive oxygen species (ROS), after which disulfides can be converted into thiols by reducing agents [9]. Thiol/disulfide redox state is important for the activity and stability of proteins, apoptosis, and as a defense mechanism against ROS [10,11]. Altered thiol/disulfide balance has been found to be associated with the etiopathogenesis of a variety of diseases [12-14].

Until recently, there were no methods available for colorimetrically measuring the thiol-disulfide balance [15]. Today, thiol and disulfide levels can be analyzed with the novel colorimetric method of Erel and Neselioglu [16].

Hence, we investigated the thiol/disulfide homeostasis in FMF patients, besides its correlation with the levels of inflammatory markers including white blood cell (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fibrinogen.

Material and Method

In this cross-sectional study, a total of 60 FMF patients diagnosed in accordance with the Tel-Hashomer Criteria [17] and 36 healthy controls were enrolled. FMF patients were divided into two groups as 33 patients during attack-free periods (AFP) and 27 patients during attack period (AP). Diagnosis of attack period was based on the presence of clinical findings such as fever, serositis/arthritis, and skin rash and elevated inflammatory markers. Demographic features and laboratory results of all three groups were recorded. Patients with obesity, diabetes, kidney and liver failure, hematologic diseases, or any malignancy, as well as pediatric patients, pregnant patients, and cigarette/alcohol/drug users were excluded from this study. The local ethics committee has approved the protocol of this study.

Sample collection and analysis

Blood samples for measuring thiol/disulfide homeostasis tests were collected from patients and healthy controls after an 8 to 12 hour fasting period. All blood samples were quickly centrifuged at 1500g for 10 min. The serum samples were then immediately stored at −78°C until assayed. Then, thiol/disulfide profile parameters were analyzed with the novel colorimetric method of Erel and Neselioglu. Also, the (disulfide x100)/native thiol, the (disulfide x100)/total thiol, and (native thiol x100)/total thiol ratios were calculated [16]. The CRP, ESR, fibrinogen levels, and WBC counts were immediately tested using classical methods.

Statistical Analysis

Data analysis was performed using SPSS 22 (SPSS Inc., Chicago, IL, USA). Data normality was examined with the Shapiro-Wilk test. Qualitative data were evaluated using x^2 test, while quantitative data were tested using the Kruskal-Wallis and Mann-Whitney U tests. Correlation analysis was evaluated with Spearman test. Data were expressed as mean ± SD or number (%) or median (min–max). $P<0.05$ was accepted as statistically significant.

Results

The study included 27 (10F, 17M) FM-AP patients, 33 (12F, 21M) FMF-AFP patients, and 36 (14F, 22M) healthy controls. Mean ages were 30.9±10.2 years [median 30 (18–58)] in the FMF-AP patients, 29.9±9.7 years [median 28 (18–51)] in the FMF-AFP patients, and 30.9±10.7 years [median 28 (17–55)] in the healthy controls.There was no marked difference in terms of age and gender distribution among the three groups. Demographic characteristics of the FMF patients are shown in Table1.

Table 1. Demographic features of the studied familial Mediterranean fever patients (FMF)

	FMF patients (n:60)
Age (years)	30.4 ± 9.9
Gender (M/F)	38/22
Age at diagnosis (years)	22.8 ± 9.4
Age at onset (years)	13.4 ± 8.7
Disease duration (years)	16.9 ± 11.3
Family history of FMF, n (%)	43 (71.7)
Previous abdominal surgery, n (%)	16 (27.6)
Colchicine dose (mg/day)	1.25 ± 0.55

Data are shown as Mean ± SD or number (%).

Laboratory results of the two FMF groups and the healthy controls are presented in Table 2. The total and native thiol concentrations of the FMF-AP group were significantly lower than those of healthy controls (p=0.016; p=0.003, respectively), (Figures 1 and 2). However, no significant difference was determined regarding disulphide levels and the ratios of thiol/disulphide homeostasis parameters. Thiol/disulfide profile parameters in the FMF-AFP group were similar to the levels in both the FMF-AP group and healthy controls.

The WBC count, CRP, fibrinogen, and ESR levels were markedly higher in the FMF-AP compared to those in the FMF-AFP group (Table 2).

We also evaluated the correlation of the thiol/disulfide profile parameters with the inflammatory markers in the FMF groups. In the FMF-AP group, total and native thiol concentrations were correlated negatively with fibrinogen (r=-0.457, p=0.028 and r=-0.440, p=0.035, respectively), while disulfide levels were correlated positively with WBC count (r=0.449, p=0.028). In the FMF-AFP group, no significant correlations were identified between thiol/disulfide profile parameters and inflammatory markers.

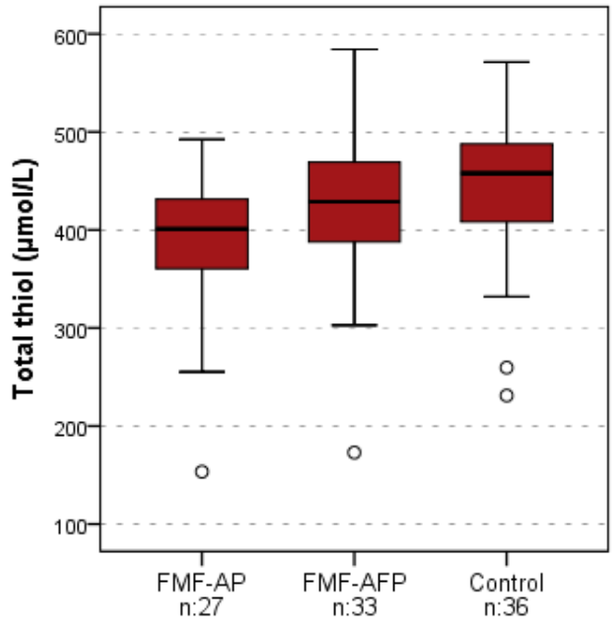

Figure 1. Total thiol levels of FMF patients and controls. Serum total thiol levels were significantly lower in patients with FMF-AP compared to healthy controls (p=0.016).

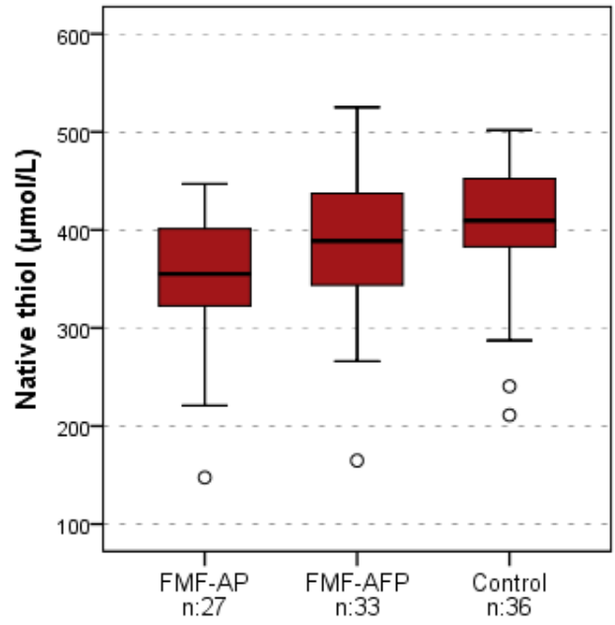

Figure 2. Native thiol levels of FMF patients and controls. Serum native thiol levels were significantly lower in patients with FMF-AP compared to healthy controls (p=0.003).

Table 2. Comparison of laboratory data of the study groups

	FMF-AP (n:27)	FMF-AFP (n:33)	Control (n:36)	P
Native thiol (µmol/L) *†	355.2 (147.5-446.9)	388.7 (164.8-525.2)	409.4 (211.1-501.8)	0.004
Total thiol (µmol/L) *†	401 (153.4-492.4)	428.9 (173-584.2)	457.8 (231.1-571.3)	0.020
Disulfide (µmol/L)	20.8 (3.0-39.8)	21.1 (4.1-34.4)	16.6 (6.9-38.2)	0.336
Disulfide/native thiol (%)	6.2 (0.8-11.8)	5.7 (2.2-11.2)	4.5 (1.7-8.5)	0.095
Disulfide/total thiol (%)	5.5 (0.8-9.6)	5.1 (2.1-9.2)	4.1 (1.7-7.2)	0.095
Native thiol/total thiol (%)	88.9 (80.9-98.5)	89.7 (81.7-95.7)	91.8 (85.5-96.7)	0.095
WBC (x10^3/µl) ‡	7.7 (6.0-16.9)	7 (3.9-11.7)	-	<0.001
CRP (mg/l)‡	56.1 (6.6-187)	3.3 (3.2-10.9)	-	<0.001
ESR (mm/h) ‡	27 (9-110)	11 (2-35)	-	<0.001
Fibrinogen (mg/dl) ‡	446 (281-759)	296 (224-432)	-	<0.001

Data are shown as median (min–max). FMF-AP= Familial Mediterranean fever patients in attack period. FMF-AFP= Familial Mediterranean fever patients in attack-free period. CRP= C-reactive protein, ESR= Erythrocyte sedimentation rate, WBC= White blood cell. p<0.05 is considered significant for statistical analyses.
*p < 0.05 for FMF-AP vs healthy controls.
†p > 0.05 for FMF-AP vs FMF-AFP, and for FMF-AFP vs healthy controls.
‡p < 0.05 for FMF-AP vs FMF-AFP.

Discussion

FMF is an autoinflammatory disease characterized by periods of exacerbation and remission. During periods of attack, a high level of inflammation with elevated levels of pro-inflammatory cytokines and increased acute phase reactants is observed in FMF patients [18,19]. Subclinical inflammation may continue during the attack-free period that occurs in the intervals between acute attacks [20].

During inflammation, the OS resulting from the excessive production of free radicals and/or reduced antioxidant defense mechanisms may lead to lipid, protein, and nucleic acid damage in the organism [21]. Lipid peroxidation (e.g., conjugated diene and malondialdehyde), protein oxidation (e.g., protein carbonyl), and DNA oxidation markers are used to identify oxidative damage [6,21]. Therefore, these markers are measured to examine the role of OS in the etiopathogenesis of many diseases, including FMF [6,8,22]. Kirkali et al. studied 17 FMF patients and showed marked accumulation of cytotoxic and mutagenic DNA lesions due to overproduction of ROS in polymorph nuclear leukocytes of FMF patients compared to controls [22]. In another study, Ediz et al. found that the protein carbonyl and malondialdehyde levels were significantly higher, and that antioxidant markers, including catalase and glutathione peroxidase levels, were markedly lower in the FMF-AP group compared with healthy controls [8]. Guzel et al. similarly reported high levels of protein carbonyl and conjugated diene, as well as low glutathione peroxidase activity, in FMF patients [6]. These results indicate that FMF is associated with increased OS.

Thiol plays a critical role in the neutralization of reactive oxygen molecules. Thiol-disulfide exchange occurs in case of ROS exposure [9]. Therefore, thiol and disulfide levels may indirectly indicate OS levels. One study reported that total thiol level was markedly higher in the FMF-AFP group compared to healthy controls. However, we could not reach any information about the relationship between high thiol levels and protective or compensatory mechanisms [23]. Guzel et al. reported that thiol levels were similar between FMF patients and controls [6]. In contrast, Omma et al. reported that total and native thiol concentrations were lower, while disulfide concentrations were higher in the FMF-AP group compared to the healthy controls [14]. Similarly, we determined that total and native thiol concentrations of FMF-AP group were significantly lower than those of healthy controls. On the other hand, disulfide concentrations were higher in FMF-AP group but there was no statistical difference. Low thiol levels are expected, since FMF is associated with OS. Thiol groups

may play a role in the defense mechanism against OS in the FMF patients, especially during the attack period. Therefore, decreased thiol concentrations may be an indicator of OS in patients with FMF-AP.

In previous studies, increased levels of CRP, ESR, and fibrinogen were detected in the patients with FMF-AP compared to the FMF-AFP group [6,24]. Similarly, we found that CRP, ESR, fibrinogen levels, and WBC count were markedly higher in the FMF-AP group. In the FMF-AP group, fibrinogen levels were found to be negatively correlated with both total thiol and native thiol, and a positive correlation was observed between the WBC count and disulfide. In a recent study, Omma et al. found that CRP and ESR levels in FMF patients correlated negatively with total and native thiol, and positively with disulfide [14]. These results indicate that thiol and disulfide levels may be linked to inflammation.

Colchicine is the principal therapy for FMF patients; it suppresses inflammation by reducing release of IL-1β, which is involved in the inflammatory process [25]. In our study, all patients received colchicine. Therefore, the effects of colchicine should be investigated by future studies that include newly-diagnosed FMF patients who are not using this medication.

Limitations

Firstly, this is a cross-sectional study of thiol and disulfide levels in FMF patients. The parameters should also be assessed during the attack and attack-free periods of the same patients. Secondly, this study was carried out at a single center and with a small sample size.

Conclusions

As a conclusion, decreased concentrations of total and native thiol in patients with FMF-AP are likely to be an outcome of inflammation-induced OS. However, to understand the importance of thiol/disulfide balance in FMF patients, it will be necessary to conduct studies with larger sample sizes.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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